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Responses of microbial residues to simulated climate change in a semiarid grassland

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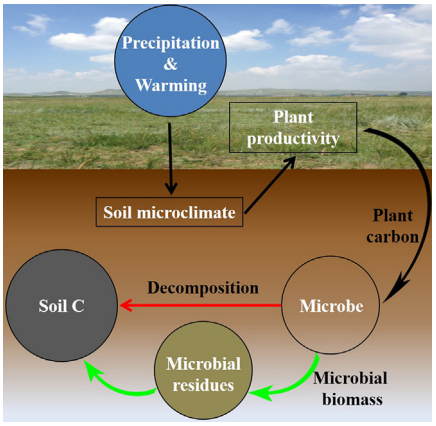
HIGHLIGHTS

- Increased precipitation and warming affected microbial residues in opposite ways.
- Changes in microbial residues were mainly determined by fungal residues.
- Altered plant productivity was highly related to changing microbial residues.
- Microbial residues had a greater response to climate change than total soil C.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial residues play important role in regulating soil carbon (C) turnover and stability, but the responses of microbial residues to climate change are neglected. In this study, a 5-year field experiment that simulated two climate change factors (precipitation and warming) was performed to examine microbial residue changes in a semiarid grassland, with water limitation. Both the contents of total amino sugars (a biomarker of microbial residues) and glucosamine (a biomarker of fungal residues) increased significantly with increased precipitation and decreased under warming, whereas neither increased precipitation nor warming influenced the content of muramic acid (a biomarker of bacterial residues). These findings clarified the role of fungal residues in determining the response of microbial residues to altered water availability and plant productivity induced by increased precipitation and elevated temperature. Interestingly, microbial residues had a much greater response to climate change than total soil C, implying that soil C composition and stability altered prior to soil C storage and simultaneously slowed down the change of soil C pool. Integrating microbial residues into current climate-C models is expected to enable the models to more accurately evaluate soil C responses to climate regimes in semiarid grasslands.

1. Introduction

Global climate is undergoing warming and predicted to continue this trend in the future (Roberts et al., 2015). Global and regional

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precipitation regimes are also changing in response to climate warming (Greve et al., 2014). Specifically, two opposite precipitation patterns are observed accompanying climate warming in dry regions: to become drier or wetter (Dai, 2013; Donat et al., 2016). The divergence in current complex climate scenarios with dramatic warming and fluctuating precipitation regimes causes the uncertainty in carbon (C) budget of terrestrial ecosystems (Carvalhais et al., 2014). Soil accounts for the largest amount of stored C in terrestrial ecosystems, and a small change in soil C pool could substantially influence future climate change (Scharlemann et al., 2014; Kirk, 2016). However, empirical data exploring how microbes mediate soil C cycling during climate change are limited.

Soil microorganisms are the key regulators of soil C decomposition and sequestration (Schimel and Schaeffer, 2012; Kaiser et al., 2014). An increasing number of studies have highlighted the significance of microbial physiology or activity in regulating soil C pool under climate change (Allison et al., 2010; Frey et al., 2013; Hagerty et al., 2014; Cheng et al., 2017) due to plant C inputs and soil C emissions (Rubino et al., 2010; Pries et al., 2017). Essentially, microorganisms can mediate soil C turnover through the processes of nutrient uptake, microbial growth and biomass formation, and the final formation of residues. Microbial residues turn over more slowly than previously thought and have a high potential to incorporate microbial production into soil C pool by microbial pathway (Lauer et al., 2011; Kögel-Knabner, 2017), especially are stabilized into soil C pool by biochemical and physical interactions (Cotrufo et al., 2015; Liang et al., 2017). However, little information has been obtained on how climate change influences microbial residues despite their great contributions to soil C pool. A clarification of microbial residue changes will be beneficial for evaluating soil C responses to future climate change scenarios.

To date, it has been difficult to directly quantify microbial residues in soil. Alternatively, amino sugars (AS) are the main constituents in microbial cell walls and can be the appropriate agents for evaluating the changes in microbial residues in soil (Glaser et al., 2004; Joergensen, 2018). As the biomarker of microbial residues, each of the AS detected in soil originates from different microorganisms: glucosamine (GluN) is mainly derived from fungi; while muramic acid (MurA) is uniquely synthesized by bacteria (Guggenberger et al., 1999; Amelung, 2001; Liang et al., 2015; Shao et al., 2017). The origin of galactosamine (GalN) is not yet clear (Engelking et al., 2007), and thus, little information on GalN is described in this study.

Semiarid grassland is susceptible to global climate change (Christensen et al., 2004; Wilcox et al., 2017), and water is the predominant limiting factor for plant productivity and soil microbial activity (Allison and Treseder, 2008; Liu et al., 2009; Zhang et al., 2015). Because it is commonly considered that fungi are more resistant to aridity than bacteria due to the capacity of fungal hyphae to take up water (Gordon et al., 2008; Barnard et al., 2013), we hypothesized that the content of bacterial-related residues was more sensitive to warming/increased precipitation than fungal-related residue content. Therefore, a study was conducted to examine the changes in microbial residues of surface soils (0–10 cm) after a continuous 5-year of field experiment with increased precipitation and warming in a semiarid grassland. Specifically, the objectives were (1) to investigate microbial residue responses to increased precipitation and warming, (2) to detect whether climatic factors effects on fungal and bacterial residues were consistent, and (3) to evaluate microbial residue contribution to soil C pools under climate change.

2. Materials and methods

2.1. Study site, experimental design and sampling

The experiment was established in April 2005 in a temperate steppe located in Duolun County (42°02′N, 116°17′E), Inner Mongolia. From 1952 to 2009, the mean annual temperature was 2.1 °C, and the mean annual precipitation was 382.3 mm (mainly distributed from May to October, as the growing season). Dominant plant species consisted of grasses (*Stipa krylovii*, *Agropyron cristatum*, and *Cleistogenes squarrosa*) and forbs (*Artemisia frigida* and *Potentilla acaulis*). The sandy soils of the experimental site were classified as Haplic Calcisols, and the pH was 7.00 ± 0.04.

The details of the experimental design were described in a previous study (Niu et al., 2011). A split-plot design was performed with two climate change factors (i.e., precipitation and warming); four treatments including control, increased precipitation, warming, and increased precipitation plus warming; and six replicates per treatment. In July and August each year, a total of 120 mm of water was evenly added to the increased precipitation plots with 15 mm per week, which accounted for approximately 30% of the mean annual precipitation in this study site. In each 3 m × 4 m warmed plot, an infrared radiator (Kalglo Electronics Inc., Bethlehem, PA, USA) was suspended 2.5 m above the ground to continuously heat from March 15 to November 15 each year. A non-heating equipment in the non-warmed plot with the same shape and size as a dummy infrared radiator was used to simulate the shading effect of the heater in the warmed plot. A CR1000 data logger (Campbell Scientific, Logan, UT, USA) was used to record soil temperature at a depth of 10 cm every hour starting in June 2005. Soil moisture at a depth of 10 cm was measured two or four times per month using a portable soil moisture probe (Diviner-2000, Sentek Pty Ltd., Balmain, Australia) from May to October during 2005–2009.

Soil samples were collected from the surface soil (0–10 cm) of all 24 plots in August 2009. In each plot, three soil cores (5 cm in diameter) were collected and mixed thoroughly as one sample. Soil samples were stored at ~0 °C and shipped to the laboratory. All the soil samples were sieved with a 2-mm mesh to remove stones, residues and thick roots, and subsampled for analyses as described below.

2.2. Total soil carbon content, aboveground biomass, microbial biomass carbon and soil respiration

The content of total soil C was determined by combustion analysis of the air-dried soil (sieved to 0.15 mm) using a Vario MACRO Cube (Elementar, Langenselbold, Germany).

Two 1 m × 0.15 m sample strips in each plot were clipped during the peak growing season in mid-August every year to estimate the aboveground living plant biomass. The vegetative tissues harvested were oven-dried at 65 °C for 48 h and weighed.

Microbial biomass C (MBC) in soil was measured by a chloroform fumigation extraction method (Vance et al., 1987b). Briefly, 5 g fresh soil from each sample was exposed to chloroform and fumigated for 24 h. After removing the chloroform, the fumigated soil was extracted using 30 ml of 0.5 M K_2SO_4. The non-fumigated soil samples were extracted simultaneously. The organic C of the extracts was analyzed using a TOC/TN Analyzer (Multi N/C 3000, Analytic Jena, Germany). The MBC was calculated as the equation: $MBC = [C_{org}\ (fum) - C_{org}\ (non\text{-}fum)] / 0.45$ (Vance et al., 1987a).

Soil respiration was measured twice per month using a Li-8100 portable soil CO_2 fluxes system (Li-Cor Inc., Lincoln, NE, USA) from May to October during 2005–2009.

2.3. Amino sugar analysis

Amino sugars (i.e., GluN, GalN, and MurA) were analyzed according to a modified method by Liang et al. (2012) based on the previous protocol of Zhang and Amelung (1996). Approximately 0.15 g of the air-dried soil sample (containing ≥0.3 mg N) was hydrolyzed with 6 M HCl (10 ml) at 105 °C for 8 h. The hydrolysate with 100 μl internal standard (myo-inositol) was filtered, dried via rotary evaporation and purified by KOH neutralization. After completely drying the solution by rotary evaporation at 52 °C under vacuum, 5 ml absolute methanol was added to dissolve the residues that were transferred to a vial and

dried by N_2 gas at 45 °C. The residue was redissolved using 1 ml deionized water, with an additional 100 μl recovery standard (*N*-methylglucamine) and subsequently freeze-dried.

To obtain the aldononitrile derivatives, a derivatization reagent containing 32 mg ml^{-1} hydroxylamine hydrochloride and 40 mg ml^{-1} 4-dimethylamino-pyridine in pyridine-methanol (4:1 v/v) was added to dissolve the lyophilized AS residues. And the solution was then shaken and heated at 75–80 °C for 35 min. After cooling, the solution with 1 ml acetic anhydride was reheated at 75–80 °C for 25 min for acetylation. At room temperature, 1.5 ml dichloromethane and 1 ml 1 M HCl were successively added, and the mixture was vortexed for 30 s to separate the organic phase. The organic phase was washed with 1 ml deionized water three times to thoroughly remove the residual anhydride. The remaining organic phase was dried by N_2 gas at 45 °C. Finally, the AS derivative dissolved with 200 μl ethyl acetate-hexane (1:1) was analyzed on an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5 column (30 m × 0.25 mm × 0.25 μm) and flame ionization detection. The AS extract (1 μl) was injected onto the column using N_2 as the carrier gas at a constant flow rate of 0.6 ml min^{-1}. The gas chromatography (GC) inlet was set to 250 °C and operated in a split mode with a 30:1 ratio. Compared to authentic standards, the individual AS derivatives were separated by their retention times. The total soil AS content was the sum of the individual AS.

2.4. Data analysis

We tested whether increased precipitation, warming and their interactions affected the soil microclimate, aboveground biomass, MBC, soil respiration, and the contents of total soil C and AS by two-way analysis of variance. Regression analysis was used to detect Pearson's linear correlations of amino sugar parameters including total AS, GluN and MurA to soil temperature, soil moisture and aboveground biomass. These statistical analyses were performed using SAS V.8.1 software (SAS Institute Inc., Cary, NC, USA). Precipitation-induced changes (%) in AS parameters and total soil C were calculated as [100 × (P − Control) / Control + 100 × (PW − W) / W] / 2; and warming-induced changes (%) were calculated as [100 × (W − Control) / Control + 100 × (PW − P) / P] / 2.

3. Results

3.1. Soil microclimate, aboveground biomass, microbial biomass carbon and soil respiration

After 5-year of field experiment, increased precipitation had no effect on soil temperature but significantly stimulated soil moisture by 1.65% ($P < 0.001$; Tables 1, 2). Increased precipitation significantly enhanced aboveground biomass (i.e., plant productivity) by 31.67% ($P < 0.001$), MBC by 16.79% ($P < 0.01$) and soil respiration by 33.65% ($P < 0.001$) (Tables 1, 2). Soil temperatures were significantly increased by 1 °C and 0.88 °C in response to warming in ambient and increased precipitation, respectively (Tables 1, 2). Warming significantly decreased soil moisture by 0.73% ($P < 0.001$), aboveground biomass by 22.51% ($P < 0.001$), MBC by 12.06% ($P < 0.05$) and soil respiration by 11.32% ($P < 0.001$) (Tables 1, 2). However, there were no interactive effects of increased precipitation and warming on soil microclimate, aboveground biomass, MBC and soil respiration (Tables 1, 2).

Table 2
Two-way analysis of variance of increased precipitation and warming effects. Statistical analysis (*F*-test and *P*-test) was performed for soil microclimate (soil temperature, ST; soil moisture, SM), aboveground biomass (AGB), microbial biomass carbon (MBC), soil respiration (SR), amino sugar parameters including total amino sugar (total AS), glucosamine (GluN), galactosamine (GalN) and muramic acid (MurA), total soil carbon (total C), and total AS to total soil C ratio (total AS/total C). Bold values represent $P < 0.05$, and the italics represent marginal significance ($P < 0.1$). The symbol of ^ represents statistical signifiance with marginal significance.

Variable	Precipitation		Warming		Precipitation × warming	
	F ratio	*P* value	*F* ratio	*P* value	*F* ratio	*P* value
ST	0.20	0.660	**33.34**	**<0.001**	0.07	0.801
SM	**468.47**	**<0.001**	**91.23**	**<0.001**	2.28	0.146
AGB	**17.05**	**<0.001**	**14.68**	**0.001**	0.24	0.631
MBC	**8.60**	**0.008**	**5.98**	**0.024**	0.48	0.498
SR	**142.77**	**<0.001**	**23.07**	**<0.001**	0.98	0.333
Total AS	**9.02**	**0.007**	**7.27**	**0.014**	0.17	0.680
GluN	**10.96**	**0.004**	**6.07**	**0.023**	0.30	0.588
GalN	**5.51**	**0.029**	**9.04**	**0.007**	0.02	0.884
MurA	0.67	0.421	0.93	0.347	0.24	0.630
Total C	4.00	*0.059^*	1.63	0.216	0.48	0.498
Total AS/total C	3.03	*0.097^*	**5.37**	**0.031**	0.07	0.800

3.2. Changes in the contents of amino sugars and total soil carbon under increased precipitation and warming

The contents of total AS, GluN and GalN increased significantly in response to increased precipitation (all $P < 0.05$), but no change was found in MurA content (Fig. 1a and Table 2). Both total soil C content and the proportion of AS to total soil C (total AS/total C) marginally responded to increased precipitation (both $P < 0.1$; Fig. 1b and Table 2). We found significant decline in the contents of total AS, GluN and GalN under warming (all $P < 0.05$), but no change in MurA content (Fig. 1a and Table 2). Warming did not influence total soil C, while AS/total C decreased significantly under warming ($P < 0.05$) (Fig. 1b and Table 2). No interactions of increased precipitation and warming on AS parameters and total soil C were found (Fig. 1 and Table 2). We also detected the relative change (%) in AS and total soil C induced by increased precipitation and warming (Fig. 2). The relative changes followed the order GluN > total soil C > MurA (Fig. 2).

3.3. Relationships of amino sugar parameters to microclimate and plant productivity

Correlation analysis was performed between AS parameters and, microclimate and plant productivity. The total AS content was negatively related to soil temperature ($r = -0.68$, $P < 0.001$; Fig. 3a), but positively related to both soil moisture and aboveground biomass ($r = 0.73$, $P < 0.001$ and $r = 0.89$, $P < 0.001$, respectively; Fig. 3b, c). In addition, a negative relationship was found between GluN and soil temperature ($r = -0.65$, $P < 0.001$; Fig. 3d), but positive relationships between GluN and, soil moisture and aboveground biomass ($r = 0.75$, $P < 0.001$ and $r = 0.88$, $P < 0.001$, respectively; Fig. 3e, f). We did not found the relationships of MurA to soil temperature, soil moisture and aboveground biomass (Fig. 3g, h, i).

Table 1
Responses of soil microclimate in the growing season (May to October), biomass and soil respiration to increased precipitation and warming treatments (means ± 1SE). Microclimate including soil temperature (ST) and soil moisture (SM); biomass including aboveground biomass (AGB, peak aboveground biomass to estimate plant productivity) and microbial biomass carbon (MBC); soil respiration (SR). Four treatments: C, control; P, increased precipitation; W, warming; PW, increased precipitation × warming.

Treatment	ST	SM	AGB	MBC	SR
C	6.36 ± 0.15	9.25 ± 0.06	249.45 ± 19.85	534.36 ± 22.13	1.63 ± 0.03
P	6.33 ± 0.19	11.01 ± 0.09	327.65 ± 23.64	595.72 ± 22.71	2.09 ± 0.07
W	7.36 ± 0.18	8.64 ± 0.07	192.83 ± 7.11	448.56 ± 41.30	1.39 ± 0.03
PW	7.25 ± 0.14	10.17 ± 0.08	254.50 ± 15.99	547.71 ± 16.81	1.93 ± 0.02

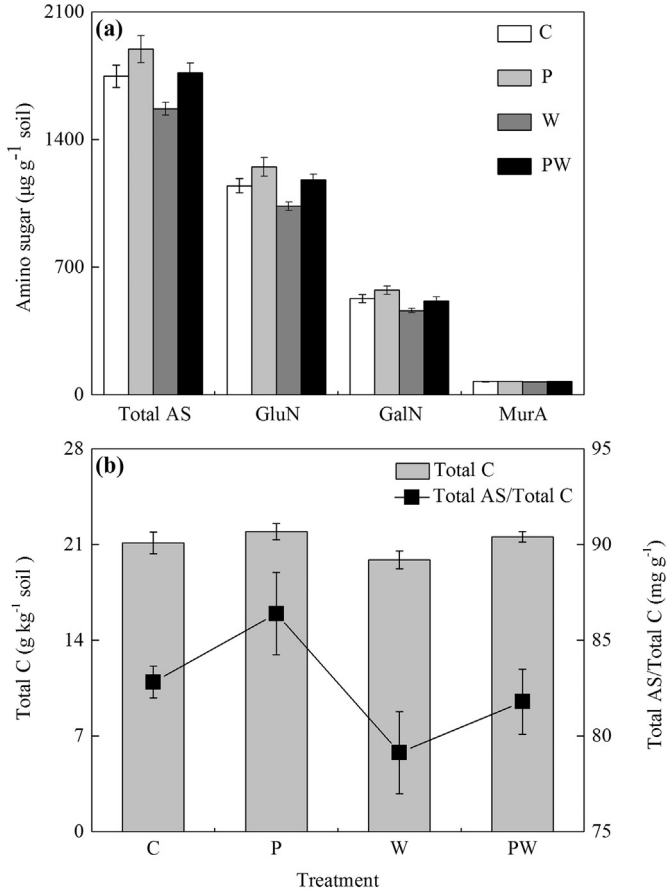

Fig. 1. Amino sugar parameters and amino sugar contribution to total soil carbon under increased precipitation and warming treatments (means ± 1SE). a, soil amino sugar parameters including total amino sugar (total AS), glucosamine (GluN) and muramic acid (MurA). b, the ratio of total AS to total soil C (total AS/total C) represents microbial residue contribution to soil C. Four treatments: C, control; P, increased precipitation; W, warming; PW, increased precipitation × warming.

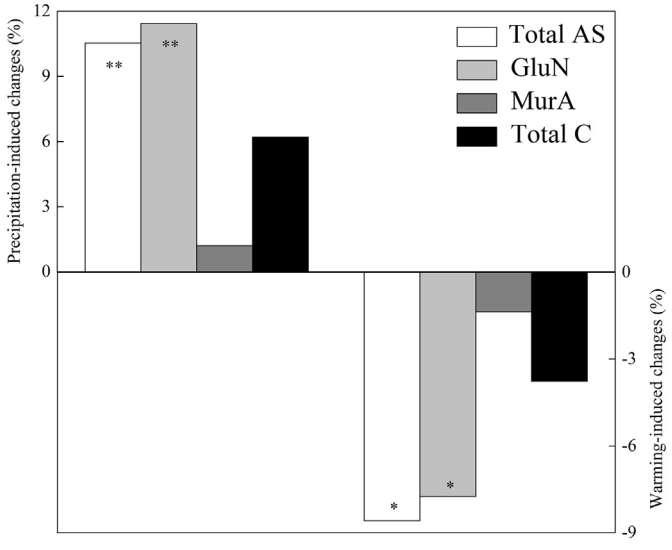

Fig. 2. Relative change (%) in amino sugar parameters and total soil carbon induced by increased precipitation and warming. Precipitation-induced changes were calculated as [100 × (P − Control) / Control + 100 × (PW − W) / W] / 2; and warming-induced changes were calculated as [100 × (W − Control) / Control + 100 × (PW − P) / P] / 2. The asterisks inside represent significant differences between increased precipitation and ambient precipitation, and the differences between warming and without warming; with $^{*}P < 0.05$, and $^{**}P < 0.01$. Abbreviation: total amino sugar (AS), glucosamine (GluN), muramic acid (MurA) and total soil carbon (total C).

4. Discussion

4.1. Responses of soil microbial residues to increased precipitation and warming

As the biomarker of microbial residues, total AS responded differently to increased precipitation and warming, suggesting that the balance of the production and decomposition of microbial residues was highly dependent on climate change factors. In this semiarid grassland, water is the predominantly limiting factor (Liu et al., 2009). The enhanced accumulation of AS under increased precipitation is likely to be representative of the preferential production of microbial residues due to the mitigation of water limitations. Soil microorganisms can be directly influenced by soil moisture and indirectly by plant productivity and plant-derived substrates (Clark et al., 2009; Sorensen et al., 2013). Because of the notably inherent resilience of microbes to fluctuations in soil moisture (Cruz-Martínez et al., 2009; Cregger et al., 2012), the shifts of soil microbes under increased precipitation are mainly explained by changed plant productivity and plant-induced-C substrates. Improved water availability increased plant productivity in this semiarid grassland (Li et al., 2017) and stimulated C inputs to soil via litter and root exudation, thus providing more active C to increase microbial biomass (Liu et al., 2009; Zhou et al., 2013). Consequently, more microbial residues accumulate in the soil with enhanced microbial metabolism from biomass to necromass.

Warming resulted in the loss of microbial residues, indicated by the reduced AS content, and particularly the decline in GluN. The exacerbated water stress caused by warming in this semiarid grassland restrains plant productivity and plant C inputs to soil (He et al., 2012). As a result, the decreased soil respiration and microbial biomass under warming indicate the low microbial growth and physiological activity. Therefore, the decreased accumulation of AS in our experiment is probably explained by the suppression of microbial biomass production related to the reduction in plant C input. Liang et al. (2015) has reported that warming reduces the accumulation of microbial residues in the California annual grassland, where the loss is considered to be due to the increased AS degradation to satisfy the N requirement of increased plant biomass under elevated temperature. The different mechanisms for decreased AS indicated the importance of limiting factors (e.g., water or N limitation) in regulating soil processes. Interactions between increased precipitation and warming did not influence microbial residues since there were no interactive effects of increased precipitation and warming on the microclimate, plant productivity and MBC via the compensatory effect of the two climate factors.

4.2. Different responses of fungal and bacterial residues to increased precipitation and warming

Climate change significantly altered fungal residues, but exhibited no influence on bacterial residues. These findings were opposite to our hypothesis, and imply that other factors induced by changing precipitation indirectly influence the response of microbial residues. In addition, the inconsistent responses of fungal and bacterial residues to precipitation and warming suggest that the responses of fungal and bacterial residues to climate change may be controlled by different mechanisms.

Our study showed that increased precipitation stimulated substantial accumulation of fungal residues. Fungi are commonly symbiotic with plants (Smith and Read, 2008; Bonfante and Anca, 2009), and water availability could establish a strong and positive link between fungi and the vegetative growth in this semiarid grassland. Improved water availability under increased precipitation induces an increase in aboveground and belowground productivity (Bai et al., 2010; Niu et al., 2011; Wilcox et al., 2017). The increased productivity results in more available C input to stimulate soil fungi, particularly plant

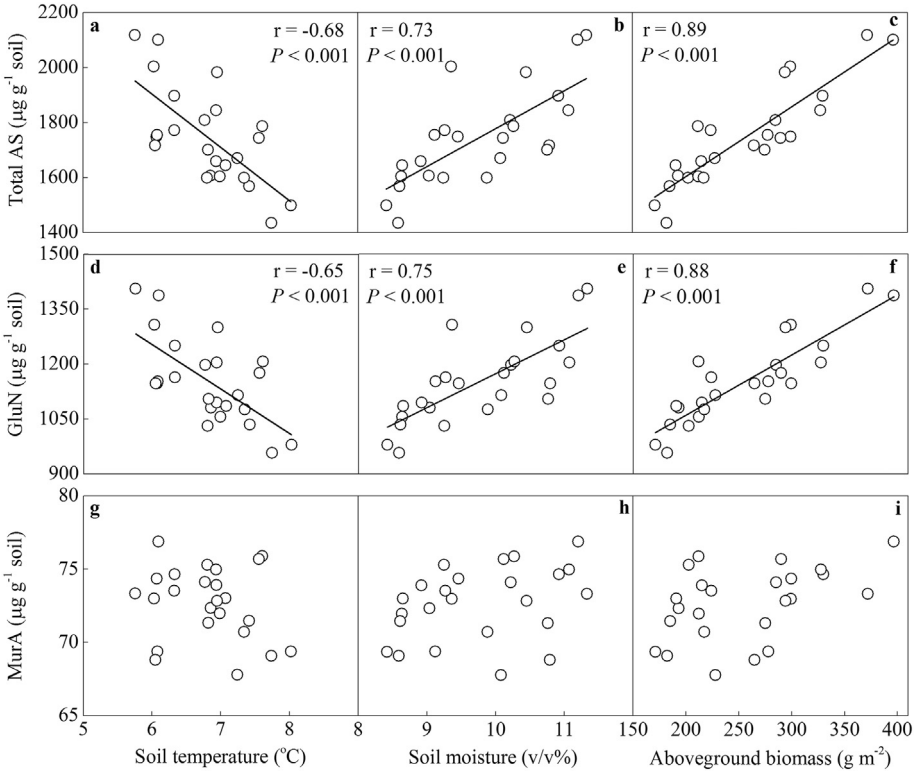

Fig. 3. Relationships of amino sugar parameters to soil microclimate and aboveground biomass. Amino sugar parameters include total amino sugar (total AS), glucosamine (GluN) and muramic acid (MurA). Solid lines depict significant linear relationships among AS parameters and predictor variables ($P < 0.05$), while plots without regression lines are not linearly related ($P > 0.05$).

symbiotic fungi, to assimilate more C into fungal biomass (Gutknecht et al., 2012; Averill et al., 2014), thus causing an increase in the formation of fungal residues. However, no change in bacterial residues was found under increased precipitation. The literatures have demonstrate that enhanced soil moisture stimulates the turnover of active bacterial communities due to increased available C (Evans et al., 2014). In addition, bacterial residues, as relatively active C components when compared to fungal residues (Nakas and Klein, 1979; Kaiser et al., 2014), can be highly decomposed by more active microbes under abundant water conditions. Thus, we hypothesize that the apparently unchanged bacterial residues under increased precipitation could be interpreted as the balance between active bacterial life cycles and bacterial residue decomposition associated with increased soil moisture and plant C input.

Inconsistent with our hypothesis, fungal residues with a substantial decline were more sensitive to warming than bacterial residues with no change. Although fungi are generally considered to be resistant to drought due to their mycelia (Gao et al., 2016), the reduced productivity caused by the exacerbated water stress under warming could restrict fungal growth and biomass due to the decline in plant C input (Compant et al., 2010). Thus, the significant decline in fungal residues under warming is the consequence of reduced fungal residues production. Despite the lower stability of bacterial residues when compared to fungal residues, the negligible change in bacterial residues under warming was not consistent with the studies that warming enhances the decomposition of stable soil carbon by lowering the activation energy (Davidson and Janssens, 2006). Two mechanisms are proposed to explain the lack of warming effect on bacterial residues: (1) low microbial activity under warming causes little degradation of bacterial residues, and (2) bacterial adaption after several years of warming, as reported by Sheik et al. (2011), could maintain the production of bacterial residues. The balance between low production and low decomposition of bacterial residues causes the apparent non-response to warming in the semiarid grassland.

4.3. Microbial residue contributions to soil carbon pool experiencing climate change

To explore the mechanisms driving microbial process during SOC cycling, the ratio of AS to total soil C was applied to indicate microbial residue contributions to soil C pool experiencing climate change (Liang et al., 2015). In this semiarid grassland, increased precipitation enhanced microbial residue contributions to soil C pool, while warming significantly reduced microbial residue contributions, especially that of fungal residues. This pattern was attributed to the greater response of microbial residues to climate change than total soil C. These findings indicate that microbial response to climate change could result in the change of soil C quality and stability prior to soil C storage in short-term considering that microbial residues contribute approximately 50% of soil C in grasslands (Khan et al., 2016; Joergensen, 2018). Different from the sensitive response of fungal residues to global change, the content of bacterial residues remained constant, implying that labile bacterial residues may play more essential role in mitigating the change of soil C pool than relatively resistant fungal residues.

5. Conclusions

Amino sugars, as a biomarker of microbial residues, allow us to explore the responses of microbial-derived C to simulated global climate change. Increased precipitation stimulated the accumulation of microbial residues, whereas warming resulted in the loss of microbial residues. Significant responses of fungal residues to precipitation and warming are ascribed to the strong dependence of fungi on plant productivity; however, no changes in bacterial residues are mainly due to fast turnover of bacteria under precipitation and its adaption to warming. The greater responses of microbial residues than soil C content to climate change suggest that soil C composition and stability alters prior to soil C storage. We expect to integrate microbial residues

into current climate-C models for more accurate predictions of soil C responses to unpredictable climate regimes in the semiarid grasslands.

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